Intraperitoneal administration of Telmisartan prevents postsurgical adhesion band formation

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Intraperitoneal administration of Telmisartan prevents post-surgical adhesion band formation. 'Revised'

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Abstract

Background: Angiotensin II receptor blockers (ARBs) have a potential role in reducing inflammation and fibrosis. We have integrated systems and molecular biology approaches to investigate the therapeutic potential of ARBs in preventing post-surgical adhesion band formation.

Material and method: we have followed the ARRIVE guidelines point by point during experimental studies. Telmisartan (1 and 9 mg/kg), valsartan (1 and 9 mg/kg) and Losartan (1 and 10mg/kg) were administered intraperitoneally in different groups of male albino Wistar rat. After 7 days of treatment, macroscopic evidence and score of fibrotic bands based on scaling methods was performed. Moreover, the anti-inflammatory and anti-fibrosis effects of Telmisartan on reduction of fibrotic bands were investigated by using histopathology, ELISA, and Real time PCR methods.

Result: Telmisartan, but not Losartan or Valsartan, prevented the frequency as well as the stability of adhesion bands. Telmisartan appears to elicit anti-inflammatory responses by attenuating sub-mucosal edema, suppressing pro-inflammatory cytokines, decreasing pro-inflammatory cell infiltration, and inhibiting oxidative stress at the site of peritoneal surgery. We also showed that Telmisartan prevents fibrotic adhesion band formation by reducing excessive collagen deposition, and suppression of pro-fibrotic genes expression at the peritoneum adhesion tissues.

Conclusion: These results support the potential application of Telmisartan in preventing post-surgical adhesion band formation by inhibiting key pathological responses of inflammation and fibrosis in post-surgery patients.

Keywords: Angiotensin receptor blockers, Telmisartan, post-surgical adhesion band formation, Inflammation, Fibrosis

Introduction

Peritoneal adhesions are pathological fibrotic bands that may develop in various conditions including endometriosis, chemical peritonitis and surgical trauma (1). Adhesion formation is a major factor causing mortality and morbidity in 90 to 95% of patients undergoing pelvic or abdominal surgery (2). Intra-abdominal adhesion contributes to increasing post-operative clinical difficulties including female infertility (3), pelvic pain (4), intestinal obstruction (5), and bleeding (6). Although, some barrier/separation materials, for example, Seprafilm and Interceed (FDA approved), as well as other strategies such as administration of anti-inflammatory, fibrinolysis and antibiotic drugs, are clinically accessible; however, these interventions are far from ideal and result in limited success (7-9). A better understanding of the pathogenesis and cellular and molecular mechanisms responsible for post-operative adhesions formation would help to develop the effectiveness of the treatment methods.

Surgical trauma or damage to the intra-abdominal cavity caused by surgery increases the secretion of cytokines contributing to the migration of inflammatory cells and fibroblast to injured peritoneum (10). Activated fibroblasts and mesothelial cells release pro-fibrotic transforming growth factor β (TGF-β) in peritoneal cavity (11, 12) which has a significant role in tissue repair and wound healing processes (13). Abnormal secretion and aberrant regulation of the TGF-β/ Smad signaling pathway increase the expression of fibrotic genes and accumulation of extracellular matrix (ECM) deposition leading fibrogenesis and adhesion bands formation (14, 15).

Identifying suitable agents for preventing post-surgical intra-abdominal adhesion formation remains an important clinical need. The literature has described cross-talk between angiotensin and TGF-β/Smad signaling can affect the fibrosis in different organs. It has been shown that angiotensin II receptor blockers (ARBs) have a potential role in reducing fibrosis through Angiotensin-TGF-β1 crosstalk in heart tissue of post-myocardial infarction (MI) rats (16). Moreover, the angiotensin type I receptor (ART1) reduced the activation of the Smad pathway in

fibrosis conditions in renal injury (17). Furthermore, Uhal et al. showed that angiotensin receptor antagonists are capable of reducing Idiopathic Pulmonary Fibrosis in man (18). In the present study, we hypothesized that the administration of ARBs may potentially reduce post-surgical abdominal adhesions formation. We investigated the therapeutic effects of three of the most important ART1 antagonists including Telmisartan, Losartan, and Valsartan in post-surgical adhesion model in the rat.

Material and Methods

Materials

Telmisartan, Losartan, and Valsartan were purchased from MedKoo Bioscience (Shanghai, China). Haematoxylin and Eosin and all reagents for catalase, superoxide dismutase (SOD), Malonyl dialdehyde (MDA) and total thiol reactions were obtained from Sigma Co (Saint Louis, MO). Interferon-γ (IFN-γ), interleukin-6 (IL-6), tissue necrosis factor-alpha (TNFα) and transforming growth factor-β (TGF-β) ELISA kits were all provided from eBioscience company (San Diego, CA). RNeasy Mini kit and cDNA Reverse Transcription Kit were purchased from Qiagen Inc. (Hilden, Germany) and TaKaRa Bio (Shiga, Japan) respectively.

Animal experiment

All experimental studies we performed according to the ARRIVE guidelines **(Ref).** Albino Wistar male rats weighing 230-250 g were purchased from the Pasture Institute (Tehran, Iran). Animals were maintained in standard temperature (24 ± 2^0 C) with 60 ± 5% humidity and 12 hr light/dark cycle. All rats had free access to tap water and were fed standard rat chow. The animal experiments were accomplished by the protocol of Care and Use of Laboratory Animals from Mashhad University of Medical Sciences (MUMS). All animal procedures were carried out according to the guidelines approved by the Ethics Committee of Mashhad University of Medical Sciences.

The post-surgical adhesion band model procedure

Surgical procedure for post-surgical adhesion band formation was described by Hemadeh et al. (8). Briefly, After 7 days, the formation of intraperitoneal adhesions occurs with high probability (up to 95%) in the positive control group. Maintenance anesthesia was performed by using ketamine (15-40 mg/kg) administered intra-peritoneally during surgery (19). Then abdominal skin shaved and cleaned with Povidone-iodine before the procedure. The

abdomen was opened with a ‘U' shaped incision and cecal abrasion by surgical gauze was continued until the presence of hemorrhagic points in abrasion sites. 3-0 chromic and 3-0 nylon catguts were used to suture the abdominal muscles and abdominal skin, respectively.

Surgical groups

Forty-eight rats were divided by double-blind randomize into eight groups (n=6) as below; 1) positive control (Cnt^+) group (surgical abrasion and peritoneal adhesion treated with ARBs solvent, dimethylsulfoxide (DMSO); 2) sham group (Cnt^-) (surgical incision with no abrasion or adhesion); 3 and 4) Telmisartan Low and High Dose (TLD) and (THD) groups (surgical abrasion treated with 1 and 9 mg/kg Telmisartan, respectively) (20-22); 5 and 6) Valsartan Low and High Dose (VLD) and (VHD) groups (surgical abrasion treated with 1 and 9 mg/kg Valsartan) (23); and 7 and 8) Losartan Low and High Dose (LLD) and (LHD) groups (surgical abrasion treated with 1 and 9 mg/kg Losartan) (24). Schematic representation of the study protocol has been shown in Fig. 1A. ARBs drugs dissolved in DMSO and administered by intraperitoneal (i.p) injection for 7 days (25). Drugs administration was performed in animal room at the morning. At the end of the treatment process, rats were sacrificed and tissue samples were stored in 10% formalin or rapidly frozen in liquid nitrogen. During the experimental period, no complications including wound infection, intra-abdominal infection, bleeding or high dose toxicity was observed in the different treatment groups.

Evaluation of adhesion scores

At the end of experiments, all rats were anesthetized and laparotomy was performed via a U-shaped incision. The peritoneum was opened to evaluate intra-abdominal adhesions according to the adhesions grade criteria described by Nair et al. (26) and Leach et al. (27). Frequency and stability of the adhesion bands were analyzed in a blinded fashion (Table 1).

Hematoxylin and Eosin (H&E) and Masson’s trichrome staining

Cross-sections of adhesion tissues between the cecum and abdominal wall were obtained and preserved in formalin 10%. H&E and Masson's trichrome staining was performed to evaluate the inflammatory cells infiltration and collagen deposition as described (28).

Enzyme-Linked Immunosorbent Assay (ELISA) analysis

Tissue specimens were collected, homogenized, centrifuged in 4000rpm for 15 min, then supernatant isolated to evaluate tissue concentration of TNF-α, IFN-γ, TGF-β, and IL-6 by rat ELISA kits (Invitrogen eBioScience, German) according to the manufacturer's instructions. Briefly, the target-specific antibody has been pre-coated in the wells of the supplied micro plates. Samples, standards, or controls are then added into these wells and bind to the immobilized (capture) antibody. The sandwich is formed by the addition of the second (detector) antibody, a substrate solution is added that reacts with the enzyme-antibody-target complex to produce measurable signal. The intensity of this signal is directly proportional to the concentration of target present in the original specimen.

Oxidative stress markers analysis

For measuring MDA level 1 ml of 10% tissue homogenates were mixed with 2 mL of thiobarbituric acid (TBA), Trichloroacetic acid (TCA), and HCL solution in boiling water for 45 minutes and centrifuged for 10 minutes. Next, the absorbance at 535 nm was read and the MDA level was measured by C (M) = A⁄1.65×105 (29)

Total thiol group was estimated by DTNB (Di-Tio nitro benzoic acid) reagent. DTNB reacts with SH groups and produces the color yellow complex. In this line, 1ml of Tris-EDTA buffer (pH = 8.6) was added to tissue homogenate and the specimen absorbance read at 412 nm against Tris-EDTA buffer alone (A1). Next, 20 µl of DTNB reagents were added to this solution and incubated at room temperature for 15 minutes. Then, the sample absorbance was

measured again (A2). The absorbance of DTNB reagent was used as a blank (B). Total thiol concentration (mM) was calculated by SH (mM) = (A2-A1-B) × 1.07 / (0.05 × 13.6.) (30).

The catalase (CAT) enzyme activity was detected as described previously by Aebi et al. The rational of this method was based on the hydrolyzation of H_2O_2 in phosphate buffer, pH 7.0, and reducing absorbance at 240 nm. The enzyme activity can be estimated by the conversion of H_2O_2 to H_2O and O_2 in 1 min under standard situation (31).

SOD enzyme activity was measured with colorimetric Madesh assay using 96 micro plates. In this method, SOD in sample tissues use superoxide anion so prevent reaction between O_2^- and MTT (32).

Real-Time Polymerase-Chain-Reaction (RT-PCR)

Real-time-PCR was performed as previously described (33). Briely, Total RNA from adhesion tissues was extracted using total RNA Mini Kit (Yekta Tajhiz Azma, Tehran). Next cDNA was synthesized according to reverse transcription kit protocol (TAKARA, Dalian, China). Real-time quantitative PCR (qPCR) was performed using SYBR Green qPCR Master Mix on a LightCycler Roche. A melting analysis was performed for evaluation the specificity of the amplification. Results were normalized against GAPDH and calibrated to control group. A relative expression was quantified using the $2^{-\Delta Ct}$ method. The PCR primer sequences are shown in Table 2.

Gene expression data analysis

Alterations in the gene expression following treatment of the HT29 colon cancer cell line with 10 µl Telmisartan (LINCSCP_126712) or losartan (LINCSCP_126358) for 24 hours were extracted from iLINCS database (34, 35). Significantly up or down-regulated genes with -0.5<log (fold change)<0.5 and adjusted p-value <0.05 were subjected to Enrichr (36) for pathway enrichment analysis using Wiki Pathways library. L1000CDS2 (37) online tool was

used to identify drugs with known mode of action that results in similar gene expression signature. Pheatmap package in R was used to create heatmaps (38). STRING (39) 11.0 database was used to create a network of protein-protein interactions with selected nodes. Interaction data obtained from text-mining were excluded from the network to increase the strength of predicting interactions.

Data analysis

All the data were expressed as the means ± standard deviation (SD). Normality of our data was analyzed using Shapiro-wilk normality test. Statistical differences were determined using one way ANOVA. A P value of <0.05 was considered to be statistically significant.

Results

Telmisartan treatment decreased adhesion bands scores in a dose-dependent manner

The surgical procedure and anesthetic induction were successfully performed and all animal models survived to seven days after the second laparotomy. The representative illustration of adhesion band grades has been presented in (Fig. 1B). According to Nair (26) and Leach (27) scoring systems, we quantitatively evaluated the frequency and rigidity of adhesion bands in all groups (Fig. 1C-D). Our results showed that the median of both adhesion scores was significantly lower in THD-treated rats compared with positive control ($Cnt^{+)}$ group ($P < 0.05$). No significant differences were observed between TLD, VLD, VHD, LLD, LHD groups with Cnt+. No adhesion band was formed in the abdomen incision in Cnt^{-} group. Since there was no difference between Valsartan- or Losartan-treated groups compared to the Cnt^{+} group, we focused on the therapeutic potency of Telmisartan in the rest of the manuscript.

Telmisartan reduced tissue damage-associated inflammatory responses in the adhesion rat model

Inflammation is one of the key factors in the pathogenesis of post-surgical adhesion bands formation. To determine the effect of angiotensin II receptor antagonist, Telmisartan, on adhesion bands-associated inflammation, peritoneum adhesion tissues were stained with Hematoxylin and Eosin (H&E) to analyze the pathological responses and morphological changes in adhesion rat models. Results showed that both low (1mg/kg) and high (9mg/kg) doses of Telmisartan were able to reduce the infiltration of inflammatory cells into the injured site but a high dose of Telmisartan was much more effective (Fig. 2A).

Next, we compared the level of pro-inflammatory cytokines including IFN-γ, IL-6, and TNF-α in peritoneum adhesion tissues homogenates between the THD, and TLD with the positive control group. We showed that high dose of Telmisartan could significantly reduce IL-6 and TNF-α concentrations (p value<0.05) (Fig. 2B and C). THD decreased INF-γ level but the

difference was not statistically significant (Fig. 2D). These results showed that the protective effects of Telmisartan against post-surgical adhesion band formation could be at least partially mediated by its anti-inflammatory responses in the rat model.

Effects of Telmisartan on oxidative stress markers

To further investigate the anti-inflammatory effects of Telmisartan in post-surgical adhesion bands, the effect of this angiotensin II receptor antagonist was evaluated on the oxidative stress markers in an adhesion rat model. Consistent with previous results, Telmisartan significantly decreased the level of MDA, an oxidant marker, in TLD (4.09 ± 0.99) and THD (2.32 ± 0.54) groups compared to the Cnt^{+} group (7.25 ± 1.30) ($p<0.05$) (Fig. 3A). Furthermore, the concentrations of total thiol, as well as the activity of catalase and superoxide dismutase (SOD) enzymes, anti-oxidant markers, were measured in the peritoneum adhesion tissues homogenates in different groups. Our results showed that total thiol, catalase and SOD indexes were significantly higher in THD (1.83 ± 0.65, 0.41 ± 0.01 and 2.19 ± 0.06, respectively) and TLD cases (0.98 ± 0.20, 0.27 ± 0.06 and 1.41 ± 0.24, respectively) than the positive control group (0.2 ± 0.028, 0.16 ± 0.013 and 0.7 ± 0.23, respectively) ($p<0.05$) in tissue homogenates (Fig. 3B-D), supporting the anti-inflammatory properties of Telmisartan in post-surgical adhesion rat model.

Telmisartan prevents adhesion formation by attenuating fibrosis in the rat model

Due to the significance of fibrosis in post-surgical adhesion band formation, Masson's trichrome staining was used to assess the collagen deposition in adhesion tissue sections. We showed that high dose of Telmisartan decreased fibrosis and tissue damage significantly (Fig. 4A). Consistent with results obtained from Masson's trichrome staining we showed that the mRNA levels of collagen 3.1 and 1.1 were significantly reduced in animals receiving a high dose of Telmisartan (9mg/kg) compared to the Cnt^{+} group (Fig. 4B and C).

Furthermore, studies are supporting the role of TGF-β in fibrotic-related complications (40). Since ARBs potentially affect the TGF-β signaling pathway through angiotensin-TGF-β crosstalk, the protein level of this marker was compared in the peritoneum adhesion tissue homogenates between groups. As presented in Fig. 4D only high dose of Telmisartan reduced TGF-β concentration in tissue homogenate samples, suggesting the anti-fibrotic effects of Telmisartan in preventing adhesion band formation.

Analysis of gene expression signature of Telmisartan

Given the observed difference in the higher efficacy of Telmisartan compared to Losartan in inhibiting the formation of adhesive bands, we analyzed the gene expression signature of the Telmisartan and Losartan at 10µl in HT29 colon cancer cell line using iLINCS database (Figure 5A-C). Following pathway enrichment analysis, we observed that in Losartan treated cells, cell cycle and G1 to S transition (adjusted p-value =1.01E-04) were the most significantly enriched pathways while for Telmisartan, apoptosis-related pathways (adjusted p-value<0.05) and corticotropin-releasing hormone (adjusted p-value = 3.70e-02) were significantly observed (Figure 5B and C).

We hypothesized that such difference between the two ARB drugs may be due to the additional targeting of PPAR-γ by Telmisartan but not Losartan and Valsartan (41). To test this hypothesis, we used L1000CDS2 online tool to identify other drugs with a known mode of action that mimics gene expression signature induced by Telmisartan or Losartan (Table 3). Accordingly, we observed that for Telmisartan, the top-ranked drugs were Rosiglitazone and Pioglitazone, known PPAR-γ agonists and 15-deoxy-delta 12, 14-prostaglandin J2 which has anti-inflammatory properties. For Losartan, however, Zolanitidine (histamine H2 receptor inhibitor), Betamethasone (glucocorticoid) and Thalidomide (immunosuppressive and anti-angiogenic) were observed. These results further strengthened the hypothesis that the

difference in higher efficiency of Telmisartan over Losartan was due to agonist of PPAR-γ by Telmisartan.

We further assessed the interaction between direct targets of Telmisartan (ATGR1 and PPAR-γ) and the proteins whose expressions were altered in adhesive bands in our study including CAT, SOD, TNF, IL6, TGFβ, COL3A1 and COL1A1 using the network of protein-protein interactions (Supplementary Fig. 1). Interestingly, among different biological processes, we observed significant enrichment of regulation of cell death (GO: 0010941) with FDR = 2.07e-07 and regulation of inflammatory response (GO:0050727) with FDR=6.71e-05, suggesting that anti-adhesive properties of Telmisartan may be through modulation of inflammation and apoptosis of stromal cell in the peritoneum by affecting TGF-β and PPAR-γ proteins.

Discussion

We have evaluated the effect of three Angiotensin II receptor blockers including Telmisartan, Losartan, and Valsartan in the adhesion rat model. We showed that only Telmisartan could significantly prevent post-surgical adhesion band formation in the rat model. Further studies showed that Telmisartan could decrease the adhesion-related inflammatory responses through reduction of inflammatory cell infiltration into damage site, reducing the expression level of inflammatory cytokines, and modulating the oxidant/anti-oxidant agents. Moreover, we showed anti-fibrotic effects of Telmisartan possibly by inhibiting pro-fibrotic agents such as TGF-β and collagen expression. These findings support the therapeutic potency of Telmisartan in decreasing post-surgical adhesion formation.

Peritoneal adhesion band formation is a common complication for patients undergoing abdominal surgery (42). When the peritoneum is injured, inflammatory cells as well as fibroblasts, are recruited to the damaged area and inflammation occurs in a large range (43, 44) and the inflammatory cytokines such as IL-6 and TNF-α significantly released. Also, it has been shown that oxidative stress markers such as MDA play an important role in adhesion formation (45). Moreover, overexpression of TGF-β enhances pathophysiological activities through ECM deposition and tissue remodeling in this condition (46). Several studies are showing that the renin-angiotensin system is involved in fibrosis via inducing collagen synthesis, increasing growth factors (47, 48), and stimulating macrophages and fibroblasts to release TGF-β (47, 49).

Xuesong et al. demonstrated that Telmisartan attenuates peritoneal dialysis-induced fibrosis by decreasing TGF-β via angiotensin type I receptor (AT1R) blockade and activation of peroxisome proliferator-activated receptor-γ (PPAR-γ) in experimental rat model (50). Arab et al. showed that Telmisartan attenuated inflammation and oxidative stress indexes in inflammatory bowel disease (IBD) in colitis rat model (51). Furthermore, the anti-inflammatory effects of Telmisartan have been shown in the acute model of inflammation (52). In line with these results, we showed

that Telmisartan can effectively inhibit the formation of adhesion bands by inhibiting fibrotic and inflammatory cytokines, factors involved in tissue remodeling, and oxidant markers.

Dose estimation always requires careful consideration about the difference in pharmacokinetics and pharmacodynamics among species (53). The tolerance and metabolism of drugs in animals are different from human. Many differences in anatomy, physiology, and biochemistry between laboratory animals and humans show a different response between animal species and humans (54, 55). Selected doses were based on previous studies for Telmisartan, losartan and valsartan in rat models. Experimental studies have demonstrated that the lethal dose 50%, amount of the substance required (usually per body weight) to kill 50% of the test population, for Losartan, Telmisartan, and valsartan is about 2000, 150-200, and 600mg/kg, respectively (56-58). The doses for these drugs in our study were much lower than these concentrations. Thus, during the experimental period, none of animals were killed in different treatment groups. Moreover, no signs of unusual behavior, or organs dysfunction related to the toxicity of the drugs were observed in rats.

Investigating the affinity of angiotensin II receptor type I antagonists showed a high interaction between Telmisartan and this receptor, compared to valsartan and Losartan (59), which may be attributed to the more potent of Telmisartan in decreasing adhesion bands in the rat model. Moreover, in another study administration of Telmisartan notably diminished the inflammatory responses in pulmonary tissue, while no such effects were shown for Valsartan treatment in the same doses (60). Moreover, enrichment of the apoptosis pathway in the gene expression data raised the hypothesis that Telmisartan may balance the proliferation and apoptosis of macrophages and fibroblasts that are accumulated at the site of surgery.

Post-surgical adhesion formation is a current problem in almost all of organs such as visceral/intra-abdominal or pelvic (61), uterus (62), tendon (63), kidney and urological (64, 65) surgeries. Formation of fibrotic bands leads to organs dysfunction causing many pathological conditions for patients. In present study, Telmisartan could show therapeutic potential to

decrease pathological conditions and fibrotic bands in rat model so using this drug in clinical trials studies and combinations with standard protocol like Seprafilm or anti-inflammatory or anti-fibrotic drugs can help protective effects to prevent post-surgical adhesion formation. Further (pre-) clinical investigations should be performed to better determine the clinical significance of ARBs against adhesion band formation in post-surgical adhesion band formation during abdominal, pelvic cavity, uterus and tendons surgeries.

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Data availability statement:

Research Data are not shared.

Author Contributions

M. H. A., F. A. and M. H. designed and performed cellular and animal experiments. A. S. and S. M. H. with support from G. A. F. and M. R wrote the manuscript. F. B. and M. J. carried out in silico analysis and performed the computations. M. F., S. N. N. and A. S. measured oxidative stress markers and performed ROS generation assays. S. M. performed ELISA experiments. S. H. M. and A. A., and F. Z. analyzed data and contributed to the interpretation of the results. S. M. H. and M. K. designed the study plan and supervised the project. All authors discussed the results and contributed to the final manuscript.

Figure legend

Figure 1. Telmisartan attenuates the incidence and rigidity of peritoneum adhesion bands. A) A Schematic representation of the study protocol. B) Telmisartan administration dose-dependently reduced the adhesion bands formation, compared to other groups. (C-D) The incidence C) and rigidity D) of adhesion bands were significantly decreased by high dose of Telmisartan, compared to control (Cnt^+) group.

Figure 2. Telmisartan reduced the inflammatory responses in adhesion rat model. (A) Hematoxylin and eosin (H&E) staining of intra-abdominal adhesion tissues revealed remarkable infiltration of leukocytes (arrows) into damage site in untreated group than treated ones. Higher dose of Telmisartan represented lower morphological damages. Also, H& E staining shows that the muscularis propria (astrix) is adhered to the skeletal muscle by fibrotic tissue. (B-D) The effects of high and low dose of Telmisartan on protein concentration of IL-6 (B), TNF-α (C) and IFN-γ (D) in tissues homogenates were compared with Cnt^+ group. *p<0.05; **p<0.01.

Figure 3. The effect of Telmisartan on oxidant/anti-oxidant balance in peritoneum adhesion bands. (A-D) The concentration of MDA (A) and total Thiol (B) as well as Catalase (C) and Superoxide Dismutase (SOD) (D) enzymes activities were compared between Cnt^+, THD and TLD groups. *p<0.05; **p<0.01; ***p<0.001.

Figure 4. Telmisartan prevent fibrogenesis in adhesion rat model. (A) Masson's trichrome staining displayed a higher collagen deposition in Cnt^+ group than Telmisartan-treated groups. Collagen-type fibrosis is shown in blue fiber (Arrows). (B-C) The mRNA expression of Collagen 3.1 (B) and Collagen 1.1 (C) in adhesion tissues were quantified and compared between THD-

treated group and Cnt^+ group. (D) Telmisartan in high doses could significantly reduce TGF-β concentration in tissue homogenates. *p<0.05; **p<0.01; ***p<0.001.

Figure 5. Assessment of gene expression changes induced by Telmisartan versus Losartan. A) Heatmap representing the expression values of genes perturbed by Telmisartan and Losartan at concentration of 10μM. B) Pathway enrichment analysis of significantly altered genes by Telmisartan using WikiPathways library in Enrichr database and comparing the expression values of genes within the significant pathways in Losartan gene expression signature. C) Pathways associated with significantly altered genes following treatment of HT29 cells with Losartan versus Telmisartan treated cells. Vertical axis demonstrates the significance of enrichment and shown as –log of adjusted p-value. Adjusted P-value <0.05 was assumed to be significant.

Supplementary figure 1. Protein-protein interaction network for direct and indirect targets of Telmisartan obtained from STRING 11.0 database